UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C.20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

November 24, 2014

MEMORANDUM

Subject: Acute Toxicity Review for EPA File Symbol: 90863-R
DP Barcode: 422250
Submission#: 955264
Subject Product Name: surPHace pHresh CONCENTRATE

From: Michelle Centra, Senior Pharmacologist
Risk Assessment and Science Support Branch (RASSB)
Antimicrobials Division (7510P)

Through: Karen P. Hicks, CTT Team Leader
Product Science Branch (PSB)
Antimicrobials Division (7510P)

To: Tom Luminello, Team Reviewer
Julie Chao, Product Manager 34
Lance Wormell, Branch Chief
Regulatory Management Branch II (RMBII)
Antimicrobials Division (7510P)

Applicant: pHresh Technologies LLC., Oakville, Ontario LL6H 6R1, Canada

FORMULATION FROM PROPOSED LABEL:

Active Ingredient:	% by weight
Sulfuric Acid (EPA PC Code 078001)	19.67
Other Ingredient(s):	80.33
Total:	100.0

BACKGROUND

The applicant, Brennis Consulting Services LLC on behalf of pHresh Technologies LLC, has submitted the following acute toxicity studies in support of registration of the proposed product, surPHace pHresh CONCENTRATE (EPA File Symbol 90863-R): oral, dermal, and inhalation toxicity studies, eye and skin irritation studies, and a dermal sensitization study.

DISCUSSION AND RECOMMENDATION

The Agency has reviewed the acute toxicity studies for the proposed product, surPHace pHresh CONCENTRATE, and made the following determinations:

1. All acute toxicity studies are classified acceptable and therefore, satisfy the acute toxicity data requirements for the registration of this proposed product.

2. The assigned acute Toxicity Categories are summarized in Table 1.

Table 1. Summary of acute toxicity of surPHace pHresh CONCENTRATE

Study	MRID	Toxicity	Status
Acute Oral Toxicity	49433203	III	Acceptable
Acute Dermal Toxicity	49433204	IV	Acceptable
Acute Inhalation Toxicity	49433205	IV	Acceptable
Primary Eye Irritation	49433208	I	Acceptable
Primary Skin Irritation	49433206	III	Acceptable
Dermal Sensitization	49433207	Non-sensitizer	Acceptable

3. Agency Review of the submitted studies (Data Evaluation Records; DERs) are appended to this memorandum.

LABELING RECOMMENDATIONS: (draft label dated 7/21/14):

Based upon results of the acute toxicity studies, surPHace pHresh CONCENTRATE, a proposed, end-use hard surface disinfectant, is classified as Toxicity Category I. Accordingly, the draft label must be modified as follows:

1. Revise the Human Hazard Precautionary Statements and PPE paragraphs to read as follows:

DANGER: CORROSIVE: Causes irreversible eye damage and skin burns. Harmful if swallowed, absorbed through skin or inhaled. Do not get in eyes, on skin or on clothing. Avoid breathing vapor or spray mist. Applicators and handlers must wear coveralls over long sleeved shirt and long pants, socks, and chemical resistant footwear, protective eyewear (such as goggles or face shield), and chemical resistant gloves (such as barrier laminate butyl rubber nitrile, PV or Viton). Wash hands thoroughly with soap and water after handling and before eating, drinking,

chewing gum, using tobacco or using the toilet. Remove contaminated clothing and wash clothing before reuse.

DATA REVIEW FOR ACUTE ORAL TOXICITY TESTING (OPPTS 870.1100)

Product Manager: 34
MRID No.: 49433203

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 7, 2014
Research Project No.: 38130

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material:	surPHace pHresh CONCENTRATE, Lot/Batch# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)	
Appearance:	Slightly white colored, slightly cloudy (opaque) liquid	
Dosage:	550, 1,750 or 5,000 mg/kg; undiluted test substance	
Stability:	Room temperature; expiration date: December 9, 2014	
Species:	Albino Rat, Sprague-Dawley	
Sex:	10 Females (nulliparous and non-pregnant)	
Age:	Young adult, 8-11 weeks at experimental start	
Weight:	154-172 g at experimental start, day 1 pre-fasted weight	
Source:	Harlan Laboratories, Inc.	
Acclimation:	9-21 days	
Housing:	Individually housed in suspended stainless steel, perforated bottom cages. Litter paper was placed beneath the cage and was changed at least three times per week.	
Food:	Harlan Teklad Global 16% Protein Rodent Diet®, available *ad libitum* except during exposure.	
Water:	Filtered tap water, available *ad libitum*	
Experimental Conditions:	Temperature	19-23^{0}C (normal range: 22^0 ± 3^{0}C)
	Humidity	42-54% (normal range 30-70%)
	Light/Dark Cycle	12 hours light/12 hours dark
	Air Exchanges	11 per hour
Method:	Up-and-Down Procedure. Acute Oral Limit and Main Toxicity Test	

Dosing: Following an overnight fast, test substance was administered to the stomach using an appropriately sized syringe and stainless steel ball-tipped needle attached to an appropriate syringe. An individual dose was calculated for each animal based on its initial body weight, taking into account the density of the test substance. Following dosing, each animal was returned to its designated cage. Feed was replaced approximately 3-4 hours after dosing.

For the initial limit dose test, 5,000 mg/kg of surPHace pHresh CONCENTRATE (undiluted) was administered to one healthy female rat by oral gavage. Due to mortality in this animal, the study proceeded to the Main Test. Using the default starting level of 175 mg/kg and following the Up and Down procedure, nine additional females were dosed at levels of 550, 1,750 or 5,000 mg/kg.

For the Main Test, surPHace pHresh CONCENTRATE was administered in sequence to the animals as presented in Table 1. The decision to proceed with the next animal was based on the survival of the previous animal following dosing. Dose progression, stopping criteria, and/or LD_{50} and confidence limit calculations were determined using the Acute Oral Toxicity (Guideline 425) Statistical Program (Westat, version 1.0, May 2001).

Table 1. Individual Animal Dosing Sequence, Dose Level and Mortality Outcome

Dosing Sequence	Animal Number	Dose Level (mg/kg)	Short-Term Outcome	Long-Term Outcome
1	3101	5,000	D	D
2	3102	175	S	S
3	3103	550	S	S
4	3104	1,750	S	S
5	3105	5,000	D	D
6	3106	1,750	D	D
7	3107	550	S	S
8	3108	1,750	S	S
9	3109	5,000	D	D
10	3110	1,750	D	D

S = Survival; D = Death

Observations, Body Weights and Necropsy: Animals were observed for mortality, signs of gross toxicity and behavioral changes during the first several hours post-dosing and at least once daily thereafter for 14 days or until death occurred. Observations included gross evaluation of skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems; somatomotor activity and behavior pattern. Particular attention was directed to tremors, convulsions, salivation, diarrhea and coma. Individual body weights of the animals were recorded prior to test substance administration on Day 0 (initial) and again on Days 7 and 14 (terminal) following dosing or after death. Surviving rats were euthanized via an overdose of CO_2 inhalation at the end of the 14-day observation period. Gross necropsies were performed on all decedents and euthanized animals. Tissues and organs of the thoracic and abdominal cavities were examined post-mortem.

Results: Individual animal body weights, doses and mortality data are presented in Table 2.

Table 2. Individual Animal Body Weights, Doses and Mortality Data

Animal Number	Sex	Dose Level (mg/kg)	Body Weight (g)			Dose (mL)	Mortality	
			Initial	Day 7	Day 14		Day	Weight (g)
3102	F	175	160	185	203	0.026	E	---
3103	F	550	162	182	186	0.079	E	---
3107	F		168	194	200	0.082	E	---
3104	F	1,750	171	196	216	0.270	E	---
3106	F		172	---	---	0.270	2	162
3108	F		172	207	212	0.270	E	---
3110	F		172	---	---	0.270	2	163
3101	F	5,000	154	---	---	0.690	2	152
3105	F		168	---	---	0.750	1	162
3109	F		172	---	---	0.770	1	169

E = Euthanized via CO_2 inhalation after weighing on Day 14.

175 mg/kg (1 animal) and 550 mg/kg (2 animals) Dose Levels

All animals survived, gained body weight and appeared active and healthy during the study. There were no signs of gross toxicity, adverse pharmacological effects or abnormal behavior. No gross abnormalities were noted for any of the animals when necropsied at the conclusion of the 14-day observation period.

1,750 mg/kg Dose Level (4 animals)

Two animals died within two days of test substance administration. Prior to death, these animals were hypoactive and exhibited irregular respiration, hunched posture (Day 0, 3-5 hours post-dosing), and reduced fecal volume (Day 1 post-dosing). Gross necropsy of the decedents revealed distention of the stomach and/or intestines and discoloration (blackish color) of the stomach and/or intestines. However, the two surviving animals appeared active, healthy and gained body weight during the study period. There were no signs of gross toxicity, adverse pharmacologic effects, or abnormal behavior in these animals. In addition, no gross abnormalities were noted for the surviving animals when necropsied at the conclusion of the 14-day observation period.

5,000 mg/kg Dose Level (3 animals)

All animals died within two days of test substance administration; 2/3 female rats on Day 0 post-dosing and 1/3 female rats on Day 1 post-dosing. Prior to death, these animals were hypoactive and exhibited irregular respiration, abnormal posture (Day 0, 0.5-6.5 hours post-dosing), and/or reduced fecal volume (Day 1 post-dosing). Gross necropsy of the decedents revealed distention of the stomach and discoloration (blackish color) of the stomach and intestines.

Under the conditions of this study, the acute oral LD_{50} of surPHace pHresh CONCENTRATE is estimated to be 1,750 mg/kg (based upon an assumed sigma of 0.5 and the one dose with partial response) in female rats with an approximate 95% (Profile-likelihood) confidence interval of 731.9 mg/kg (lower value) to 4,440 mg/kg (upper value). Based on these results, the proposed hard surface disinfectant, surPHace pHresh CONCENTRATE (Lot# 140121-4806) is assigned Toxicity Category III for acute oral toxicity.

Deviations from Guideline 870.1100: None.

Summary:

1. **Estimated LD_{50}**: = 1,750 mg/kg in female rats (95% CL: 731.9 to 4,440 mg/kg)
2. **Toxicity Category**: III
3. **Classification**: Acceptable

DATA REVIEW FOR ACUTE DERMAL TOXICITY TESTING (OPPTS 870.1200)

Product Manager: 34
MRID No.: 49433204

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 7, 2014
Research Project No.: 38131

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material:	surPHace pHresh CONCENTRATE, Lot/Batch# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)
Appearance:	Slightly white colored, slightly cloudy (opaque) liquid
Dosage:	5,000 mg/kg; undiluted test substance
Stability:	Room temperature, expiration date: December 9, 2014

Species:	Albino Rat, Sprague-Dawley Derived
Sex:	5 Males; 5 Females (non-pregnant and nulliparous)
Age:	Young adult, 8 weeks at experimental start
Weight:	Males: 191-206 g; Females: 154-163 g, at experimental start
Source:	Harlan Laboratories, Inc.
Acclimation:	6 days
Housing:	Individually housed in suspended stainless steel, perforated bottom cages. Litter paper was placed beneath the cage and was changed at least three times per week.
Food:	Harlan Teklad Global 16% Protein Rodent Diet® 2016, available *ad libitum* except for overnight fast prior to dosing.
Water:	Filtered tap water, available *ad libitum*

Experimental Conditions:	Temperature	20-23^{0}C (normal range: 22^0 ± 3^{0}C)
	Humidity	44-55% (normal range 30-70%)
	Light/Dark Cycle	12 hours light/12 hours dark
	Air Exchanges	12 per hour

Test Site Preparation: On the day prior to application of the test substance, the dorsal area of the trunk of each animal was clipped free of hair to expose an area at least 2 inches x 3 inches. Only healthy, naive animals (5 males and 5 females) with exposure areas free of pre-existing skin irritation or defects were selected for testing. A single intact exposure site was selected as the test site on each animal.

Dosing: Individual doses were calculated based upon the initial body weights, taking into account the density (1.124 g/mL using statistical analysis) of the test substance. On Day 0 of the study, undiluted test substance (5,000 mg/kg body weight) was applied evenly over the 2 inches x 3 inches test site (approximately 10% of the body surface). The dosed area was covered with a 4-ply, 2-inch x 3-inch gauze patch. The gauze pad and entire trunk of each animal were wrapped with 3-inch Durapore tape to avoid dislocation of the pad and to minimize loss of the test substance. The rats were then returned to their designated cages. After 24 hours of exposure to

the test substance, gauze pads and wrappings were removed and test sites gently cleansed with a 3% soap solution followed by tap water and clean paper towel to remove any residual test substance.

Observations, Body Weights and Necropsy: Animals were observed for mortality, signs of gross toxicity and behavioral changes during the first several hours post-dosing and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur; eyes and mucous membranes; respiratory, circulatory, autonomic and central nervous systems; somatomotor activity and behavior pattern. Particular attention was directed to tremors, convulsions, salivation, diarrhea and coma. Individual body weights of the animals were recorded prior to test substance administration on Day 0 (initial) and again on Days 7 and 14 (terminal) of the study period. All rats were euthanized via CO_2 inhalation and gross necropsies performed at terminal sacrifice (Day 14). Tissues and organs of the thoracic and abdominal cavities were examined post-mortem.

Results: All animals survived the single, 5,000 mg/kg (24-hour) exposure to undiluted test substance and gained body weight during the study (Table 1). Erythema was noted at all dose sites between Days 1 and 2 (3/10 rats), Days 1 and 4 (1/10 rats), Days 1 and 6 (5/10 rats) and Days 1 and 13 (1/10 rats). Small areas of superficial eschar were also noted between Days 2 and 5 (2/5 rats), Days 2 and 7 (1/10 rats), Days 2 and 8 (2/10 rats), Days 2 and 13 (1/10 rats), Days 3 and 5 (1/10 rats), Days 3 and 6 (1/10 rats), Days 3 and 7 (1/10 rats) and Days 3 and 8 (1/10 rats). However, no clinical findings were observed during the study and gross necropsy at the conclusion of the 14-day observation period revealed no abnormalities for any animal tested.

Table 1. Individual Animal Body Weights

Animal Number	Sex	Body Weight (g)			Dose (mL)
		Initial	Day 7	Day 14	
3201	M	191	230	268	0.85
3202	M	206	227	264	0.92
3203	M	198	221	256	0.88
3204	M	201	235	268	0.89
3205	M	206	243	278	0.92
3206	F	156	172	189	0.69
3207	F	154	172	185	0.69
3208	F	163	172	182	0.73
3209	F	163	184	209	0.73
3210	F	161	177	199	0.72

Under the conditions of this study, the single dose acute dermal LD_{50} the test substance is greater than 5,000 mg/kg in male and female rats. Based on these results, the hard surface disinfectant surPHace pHresh CONCENTRATE (Lot# 140121-4806) is assigned Toxicity Category IV for acute dermal toxicity.

Deviations from Guideline 870.1100: None.

Summary:

1. **Estimated LD_{50}**: >5,000 mg/kg in male and female rats
2. **Toxicity Category**: IV
3. **Classification**: Acceptable

DATA REVIEW FOR ACUTE INHALATION TOXICITY TESTING (OPPTS 870.1300)

Product Manager: 34
MRID No.: 49433205

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 7, 2014
Research Project No.: 38132

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material:	surPHace pHresh CONCENTRATE, Lot# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)
Appearance:	Slightly white colored, slightly cloudy (opaque) liquid
Dosage:	2.09 mg/mL mg/kg; undiluted test substance
Stability:	Room temperature, expiration date: December 9, 2014

Species:	Rat, Sprague-Dawley (Albino)
Sex:	5 Males; 5 Females (nulliparous and non-pregnant)
Age:	Young adult, 10 weeks at experimental start
Weight:	Males: 266-324 g; Females: 210-231 g, at experimental start
Source:	Harlan Laboratories, Inc.
Acclimation:	26 days
Housing:	Individually housed in suspended stainless steel, perforated bottom cages. Litter paper was placed beneath the cage and was changed at least three times per week.
Food:	Harlan Teklad Global 16% Protein Rodent Diet® 2016, available *ad libitum* except during dosing.
Water:	Filtered tap water, available *ad libitum* except during exposure.

Experimental Conditions:	Temperature	20-23^{0}C (normal range: 22^0 ± 2^{0}C)
	Humidity	44-55% (normal range 30-70%)
	Light/Dark Cycle	12 hours light/12 hours dark
	Air Exchanges	12 per hour

Method:	Limit test at a dose concentration of 2.09 mg/L
Dosing:	Nose-only, four-hour inhalation exposure

Selection of Animals: On the day of and prior to exposure, the rats were examined for health and weighed. Ten healthy, naïve rats (five males and five females; not previously tested) were selected for acute inhalation toxicity testing.

Cage-Side Observations and Body Weights: All animals were observed for mortality during the exposure period and examined for signs of gross toxicity, and behavioral changes upon removal from the exposure tube and at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma. Individual body

weights were recorded prior to test substance exposure (initial) and again on Days 1, 3, 7 and 14 (terminal) of the study period.

Exposure/Study Termination and Necropsy: At termination of the exposure period, excess test substance was removed from the ventral area fur of each animal by rinsing with tap water and clean paper towel. Animals were returned to their stock laboratory cages. All rats were euthanized via CO_2 inhalation and gross necropsies performed at terminal sacrifice (Day 14). Tissues and organs of the thoracic and abdominal cavities were examined post-mortem.

Pre-Test Trials: Prior to initiation of the full inhalation study, a pre-test trial was conducted to establish generation procedures to achieve a chamber concentration of at least 2.0 mg/mL and a particle size distribution MMAD between 1 and 4 µm. The aerosolization equipment (Table 1) and procedures used in the full test were based on the results of pre-test number 1 which provided a gravimetric concentration of 2.23 mg/mL and a MMAD of 2.58 µm.

Air Supply and Generation of Test Atmosphere (Table 1): Approximately 30 Lpm of filtered generator air was supplied via air compressor measured with a Mass Flow Controller connected to the spray atomization nozzle. An additional 6 Lpm of filtered mixing air from the same air compressor was introduced into the chamber to uniformly distribute the test atmosphere by creating a vortex at the chamber inlet. The test atmosphere was generated using a nebulizer, clean-out needle, fluid cap and air cap. Compressed generator air and mixing air were supplied at 30/30 psi, respectively. The test substance was metered to the atomization nozzle through appropriately sized tubing, using a peristaltic pump.

Table 1. Atmosphere Generation System Equipment

Liquid Pump	Peristaltic pump (Masterflex®, Model #77521-50)
Tubing for Pump	Size 14 PharMed®BPT tubing
Air Supply	Air Compressor (Powerex Model: SES050822)
Atomization	JCO Nebulizer, ¼ inch (Spraying Systems Co.)
Clean-out Needle and Fluid Cap	FC3 (Spraying Systems Co.)
Air Cap	70 SS (Spraying Systems Co.)
Exposure Chamber	28 liter (Nose-Only Inhalation Chamber ADG Developments LTD)
Compressed Generator Air Measurements	Mass Flow Controller (Omega, Model #FMA-5641)
Compressed Mixing Air Measurements	Mass Flow Meter (Omega, Model #FMA-5613)
Gravimetric Airflow Measurements	Mass Flow Controller (Aalborg Model #GFC-17)
Vacuum Pump	GAST (Model #1531-107B-G557X)

Exposure Conditions (Table 2): Chamber airflow was monitored periodically during the exposure period and recorded. Total airflow was 36 Lpm. Based on the volume of the inhalation chamber, this airflow provided approximately 77 air changes per hour during exposure. Temperature and relative humidity within the exposure chamber and the exposure room were measured and recorded every 15 minutes for the first hour of exposure and approximately every 15 or 30 minutes thereafter. Chamber temperature and relative humidity ranges during exposure were 18-19^{0}C and 24-30%, respectively; whereas the room temperature and relative humidity ranges during exposure were 17-19^{0}C and 29-33%, respectively. A humidifier was used in the room during exposure.

Test Substance Administration: After establishing the desired generation procedures during the pre-test trial, ten healthy rats (5/sex) were exposed to the chamber atmosphere containing an aerosol of the test substance for 4 hours and 4 minutes. The exposure period was extended beyond 4 hours to allow the chamber to reach equilibrium (T_{99}). The times for 90% and 99% equilibration of the chamber atmosphere was 1.79 and 3.58 minutes, respectively. When 99% concentration (T_{99}) was attained, the animals which were individually housed in polycarbonate holding tubes sealed to the chamber were then inserted into the nose-only inhalation chamber for the specified exposure period.

Test Substance Concentration: Concentration of the test substance was monitored periodically during the exposure period and recorded. Gravimetric samples were withdrawn at 6 intervals for 1 minute at airflows of 4 Lpm from the breathing zone of animals. Filter papers were weighed before and after collection to determine the mass collected. This value was divided by the total volume of air sampled to determine the test substance chamber concentration.

Particle Size Distribution: An ambient particle sizing sampler was used to assess the particle size distribution of the test atmosphere. Samples were withdrawn from the breathing zone of the animals at two intervals. The filter paper collection stages were weighed before and after sampling to determine the mass collected upon each stage. The mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined graphically using two-cycle logarithmic probit axes.

Statistical Analysis: Statistical analysis was limited to the calculation of mean and standard deviation.

Results:

The gravimetric and nominal chamber concentrations were 2.09 mg/mL and 36.6 mg/L, respectively (Table 2).

Table 2. Chamber Environment

Mean Gravimetric Exposure Level (mg/L)	2.09
Nominal Concentration (mg/L) = Total Test Substance Used (mg) ÷ Average Total Airflow (Lpm) x Total Time of Exposure (min)	36.6
Total Test Substance Used (mg)	758.7
Average Total Air Flow (Lpm)	36.0
Total Time of Exposure (min)	244
Chamber Volume (L)	28
Mean Temperature in Exposure Tube (°C)	18-19
Relative Humidity (%)	24-30

The mass median aerodynamic diameter (MMAD) was estimated to be 2.35 µm based on graphic analysis of the particle size distribution as measured with a 1 ACFM Andersen Ambient Particle Sizing Sampler with and average geometric standard deviation of 2.05 µm (Tables 3 and 4).

Table 3. Particle Size Distribution

Stage	Effective Cutoff Diameter (µm)	% of Total Particles Captured (by weight)	Cumulative (%)[1]
Sample 1			
0	9.0	1.7	98.3
1	5.8	6.4	91.9
2	4.7	2.6	89.3
3	3.3	16.2	73.1
4	2.1	39.3	33.8
5	1.1	26.5	7.3
6	0.7	4.3	3.0
7	0.4	1.3	1.7
F	0.0	1.7	0.0
Sample 2			
0	9.0	2.2	97.8
1	5.8	7.8	90.0
2	4.7	3.9	86.1
3	3.3	14.9	71.2
4	2.1	37.8	33.4
5	1.1	28.7	4.8
6	0.7	3.9	0.9
7	0.4	0.0	0.9
F	0.0	0.9	0.0

[1] Percent of particles smaller than corresponding effective cutoff diameter.

Table 4. Summary of Particle Size Distribution

Sample Number	Time of Sample (hour)	Collection Time (minutes)	Mass Median Aerodynamic Diameter (µm)	Geometric Standard Deviation (µm)
1	1.5	1	2.22	2.06
2	3	1	2.47	2.03
		Average	**2.35**	**2.05**
		% of Particles < 4.7 µm	**87.5** (Average of Two Samples; 89.3 + 86.1 ÷ 2)	

There were no reported mortalities for acute inhalation toxicity; all animals survived exposure to the test atmosphere (Table 5).

Table 5. Reported Mortality

Exposure Concentration (mg/L)	Number of Deaths / Number of Animals Tested		
	Males	Females	Combined
2.09	0 / 5	0 / 5	0 / 10

Although all animals had minor body weight losses during the study, they gained body weight over the 14-day observation period. The observed body weight losses were not considered to be of toxicological significance (Table 6).

Table 6. Individual Body Weights

Animal Number	Sex	Body Weight (g)				
		Initial	Day 1	Day 3	Day 7	Day 14
3301	M	324	305	271	301	340
3302	M	299	288	274	294	317
3303	M	266	265	268	272	286
3304	M	312	289	286	296	324
3305	F	286	276	275	287	302
3306	F	231	229	213	230	241
3307	F	214	213	205	221	230
3308	F	210	210	221	223	233
3309	F	227	223	225	227	245
3310	F	214	209	216	218	222

Following exposure, all rats exhibited irregular respiration (5/5 males at time of removal from exposure tube on Day 0 to Day 4 and 5/5 females at time of removal from exposure tube on Day 0 to Day 2). In addition, two males exhibited anogenital staining (2/5 males on Day 1 to Day 3 post-dosing), three males exhibited gasping (2/5 males at time of removal from exposure tube to 1 hour on Day 0 and 1/5 males from Day 2 to Day 3 post-dosing) and two males exhibited moist rales (1/5 males at time of removal from exposure tube on Day 0 to Day 1 and 1/5 males on Day 0 to Day 1 post-dosing). However, all animals recovered by Day 5 and appeared active and healthy for the remainder of the 14-day observation period. No gross abnormalities were noted for any of the animals when necropsied at study termination.

Conclusion: Under the conditions of this study, the median lethal inhalation concentration (LC_{50}) of the proposed product, surPHace pHresh CONCENTRATE, is greater than 2.09 mg/L in male and female rats. Therefore, surPHace pHresh CONCENTRATE is assigned Toxicity Category IV for acute inhalation toxicity.

Deviations from Guideline 870.1300: None

Summary:

1. **LC_{50} (mg/L)**: > 2.09 mg/L in male and female rats
2. **Average MMAD**: 2.35 µm
3. **Toxicity Category**: IV
4. **Classification**: Acceptable

DATA REVIEW FOR ACUTE EYE IRRITATION TESTING (OPPTS 870.2400)

Product Manager: 34
MRID No.: 49433208

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 15, 2014
Report No.: 38133

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material:	surPHace pHresh CONCENTRATE, Lot# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)
Appearance:	Slightly white colored, slightly cloudy (opaque) liquid
Dosage:	2.09 mg/mL mg/kg; undiluted test substance
Stability:	Room temperature, expiration date: December 9, 2014

Species:	Albino Rabbit, New Zealand White
Sex:	3 Females (nulliparous and non-pregnant)
Age:	Young adult at experimental start
Weight:	2.345-2.806 kg at experimental start
Source:	Robinson Services, Inc.
Acclimation:	12 or 20 days
Housing:	Individually housed in suspended stainless steel, perforated bottom cages. Litter paper was placed beneath the cage and was changed at least three times per week.
Food:	Harlan Teklad Global 16% High Fiber Rabbit Diet® 2031(approximately 150 grams/day) and a Premium Timothy Cube™ (Ontario Dehy, Inc.) were available to each rabbit.
Water:	Filtered tap water, available *ad libitum* except during exposure.

Experimental Conditions:	Temperature	19-22^{0}C (normal range: 22^0 ± 3^{0}C)
	Humidity	40-52% (normal range 30-70%)
	Light/Dark Cycle	12 hours light/12 hours dark
	Air Exchanges	12 per hour

Method: Single eye instillation, 0.1 ml of undiluted test substance

Preparation and Selection of Test Animals: Prior to test initiation, both eyes of a group of animals were examined using a white light source and a fluorescein dye procedure. One drop of 2% ophthalmic fluorescein sodium was instilled into both eyes of each rabbit followed by rinsing with physiological saline (0/9% NaCl) before evaluation of corneal damage using an ultraviolet light source. Prior to test substance instillation, the eyes were re-examined and scored for abnormalities. Three healthy naïve (not previously tested) animals without pre-existing eye defects or irritation were selected for testing.

A systemic analgesic (0.1 mg/kg Buprenorphine SR®) was administered to each animal prior to test substance instillation and at appropriate intervals to maintain therapeutic blood levels. The

analgesic provided relief from eye pain/discomfort for periods up to 76 hours due to the acidic property of the test substance, Sulfuric acid (pH 1.68, as a 1% w/w solution).

Study Design: The primary eye irritation study was conducted in a step-wise fashion. Initially, only one rabbit was placed on test. Based upon the results (corneal opacity clearing by Day 14 and absence of significant irritation/corrosive effects) for the first animal, two additional animals were placed on test to confirm the result.

Instillation: Prior to instillation of test substance on study Day 0, 2-3 drops of ocular anesthetic (Tetracaine Hydrochloride Ophthalmic Solution, 0.5%) were placed into both the treated and control eye of each animal. Undiluted test substance (0.1 mL) was then instilled into the conjunctival sac of the right eye of each animal that was formed by gently pulling the lower eyelid away from the eyeball to form a cup to accommodate administration. Following instillation, the eyelids were gently held together for one second to prevent loss of test substance. Only the right eye of each rabbit was dosed while the contralateral left eye served as a control.

Ocular Scoring: Ocular irritation was evaluated using a white light source in accordance with the method of Draize *et al.* (*J. Pharmacol. Exp. Ther.* 1944; 82:377-390) at 1, 24, 48 and 72 hours and 4, 7, 10, 14, 17 and 21 days post-instillation. Corneas of all treated eyes were examined with fluorescein sodium ophthalmic solution immediately after the 24-hour observation and as needed at subsequent scoring intervals to evaluate the extent of corneal damage or to verify reversal of effects. Individual scores were recorded for each animal. Corneal involvement or iridic irritation with a score of 1or more is considered positive while conjunctival irritation (redness or chemosis) with a score of 2 or more is considered positive. In addition to observations of the cornea, iris and conjunctivae, any other observed lesions were noted.

The average irritation score for all rabbits at each scoring (time) interval was calculated to aid in data interpretation. The time interval with the highest mean score (Maximum Mean Total Score; MMTS) for all rabbits was used to classify test substance by the system of Kay and Calandra (*J. Soc. Cos. Chem.* 1962; 13:281-289). Statistical analysis was limited to the calculation of the mean irritation scores.

Body Weights and Cage-Side Observations: Individual weights of animals were recorded shortly before instillation of the test substance (initial) and at the completion of testing (terminal). Additional body weights were recorded (not provided in the study report) to determine the appropriate amount of analgesic to administer to the animals. The animals were also observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

Study Termination: According to the study investigators, animals were humanely euthanized once testing was complete.

Toxicity Category: Toxicity Category is determined by the presence and duration of corneal involvement, iridic irritation, and positive conjunctival irritation.

Results: Two rabbits (animal number 3402 and 3403) were euthanized for humane reasons on study Day 14. The surviving female rabbit (animal number 3401) appeared active and healthy. This animal gained weight through Day 21 (study termination) whereas the two animals that were euthanized on Day 14 gained only a slight amount of weight from initiation of testing (Table 1). With the exception of eye irritation, there were no other signs of gross toxicity, adverse pharmacological effects, or abnormal behavior in any animal tested.

Within 24 hours of test substance instillation, all three treated eyes exhibited corneal opacity, iritis and positive conjunctivitis. The overall incidence and severity of irritation appeared to be severe. Minimal conjunctivitis persisted for the surviving rabbit (animal number 3401) through Day 21 (study termination). Blanching was noted for two females (animal number 3402 and 3403) between 1 hour and up to Day 14 and a white milky discharge was evident for one female (animal number 3401) between 48 hours and Day 4 of the study period (Table 2).

Under the conditions of this study, the Maximum Mean Total Score (MMTS) at 24 hours post-instillation is 79.7 (Table 3). SurpHace pHresh CONCENTRATE is therefore classified as causing irreversible destruction to the ocular tissue.

Table 1. Individual Body Weights

Animal Number	Sex	Body Weight (kg)	
		Initial	Terminal
3401	Female	2.345 (Study Day 0)	2.709 (Study Day 21)
3402*	Female	2.723 (Study Day 0)	2.816 (Study Day 14)
3403*	Female	2.790 (Study Day 0)	2.812 (Study Day 14)

*Two female rabbits were euthanized prior to study termination for humane reasons; severe ocular irritation (animal number 3402) and ulceration of the cornea (animal number 3403).

Table 2. Individual Scores for Ocular Irritation

Individual Animal Eye Irritation Scores										
	Rabbit No.: 3401[1] (Female)									
	Hours				Days					
	1	**24[2]**	**48**	**72**	**4**	**7**	**10**	**14**	**17**	**21**
I. Cornea										
A. Opacity	1	2[4]	2[4]	2[4]	2[4]	2	2	0[4]	0	0
B. Area	4	4	2	2	2	2	1	4	4	4
(AxB)x5	20	40	20	20	20	20	10	0	0	0
II. Iris										
A. Values	0	2	1	1	1	1	1	0	0	0
Ax5	0	10	5	5	5	5	5	0	0	0
III. Conjunctivae										
A. Redness	1	2	2	2	2	2	1	1	0	0
B. Chemosis	2	3	3	3	3	2	1	1	1	0
C. Discharge	2	3	2[5]	2[5]	2[5]	2	1	2	1	1
(A+B+C)x2	10	16	14	14	14	12	6	8	4	2
Total	**30**	**66**	**39**	**39**	**39**	**37**	**21**	**8**	**4**	**2**
	Rabbit No.: 3402 (Female)									
	Hours				**Days**					
	1	**24**	**48**	**72**	**4**	**7**	**10**	**14[3]**	**-**	**-**
I. Cornea										
A. Opacity	3	3[4]	2[4]	2	2	2	2	2	-	-
B. Area	4	4	4	4	4	4	4	4	-	-
(AxB)x5	60	60	40	40	40	40	40	40	-	-
II. Iris										
A. Values	1	1	1	1	1	1	2	2	-	-
Ax5	5	5	5	5	5	5	10	10	-	-
III. Conjunctivae										
A. Redness	1[6]	1[6]	2[6]	2[6]	2[6]	2[6]	2[6]	2[6]	-	-
B. Chemosis	1	2	2	2	2	2	2	3	-	-
C. Discharge	0	1	3	2	2	2	2	2	-	-
(A+B+C)x2	4	8	14	12	12	12	12	14	-	-
Total	**69**	**73**	**59**	**57**	**57**	**57**	**62**	**64**	-	-
	Rabbit No.: 3403 (Female)									
	Hours				**Days**					
	1	**24**	**48**	**72**	**4**	**7**	**10**	**14**	**-**	**-**
I. Cornea										
A. Opacity	4	4[4]	3[4]	3	3	3	3	-[2]	-	-
B. Area	4	4	4	4	4	4	4	-	-	-
(AxB)x5	80	80	60	60	60	60	60	-	-	-
II. Iris										
A. Values	2	2	2	2	2	2	2	2	-	-
Ax5	10	10	10	10	10	10	10	10	-	-
III. Conjunctivae										
A. Redness	1[6]	2[6]	3[6]	3[6]	3[6]	3[6]	3	3	-	-

Individual Animal Eye Irritation Scores										
	Rabbit No.: 3401[1] (Female)									
	Hours				Days					
B. Chemosis	1	2	2	2	2	2	3	3	-	-
C. Discharge	0	1	2	2	2	2	2	3	-	-
(A+B+C)x2	4	10	14	14	14	14	16	18	-	-
Total	**94**	**100**	**84**	**84**	**84**	**84**	**86**	-	-	-

[1]Animal immediately vocalized and rubbed eye for short period of time after dosing.
[2]Animal euthanized for humane reasons; ulceration of the cornea.
[3]Animal euthanized due to termination of testing.
[4]2% ophthalmic fluorescein sodium used to evaluate the extent of corneal opacity or to verify reversal of effects.
[5]White, milky discharge.
[6] Blanching of the nictating membrane.

Table 3. Incidence and Severity of Eye Irritation

Time, Post-Installation	Incidence of Positive Effects*			Severity of Irritation (MMTS)**
	Corneal Opacity	Iritis	Conjunctivitis	
1 hour	3/3	2/3	1/3	64.3
24 hours	3/3	3/3	3/3	79.7
48 hours	3/3	3/3	3/3	60.7
72 hours	3/3	3/3	3/3	60.0
Day 4	3/3	3/3	3/3	60.0
Day 7	3/3	3/3	3/3	59.3
Day 10	3/3	3/3	2/3	56.3
Day 14	-[1]	2/3	2/3	-[1]
Day 17	0/1	0/1	0/1	4.0
Day 21	0/1	0/1	0/1	2.0

* Incidence of Positive Effects = No. of Animals Testing "Positive" / No. of Animals Tested.
**Maximum Mean Total Score (MMTS) is the calculated average of total irritation scores for all rabbits within each time interval.
[1]Unable to determine severity due to ulceration of the eye for animal number 3403.

Based on the corrosive properties of the test substance and study results (irreversible destruction of ocular tissue), Toxicity Category I for primary eye irritation is assigned to this proposed hard surface disinfectant product.

Deviations from Guideline 870.2400: Although the protocol stated that all animals will be examined using ophthalmic fluorescein sodium and scored at the 24-hour interval, animal number 3401 had the fluorescein administration and scoring of the cornea and iris at approximately 25 hours. This slight deviation in protocol did not adversely affect the outcome of this study.

Summary:

1. **Toxicity Category**: I
2. **Classification**: Acceptable

DATA REVIEW FOR ACUTE DERMAL IRRITATION TESTING (OPPTS 870.2500)

Product Manager: 34
MRID No.: 49433206

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 7, 2014
Report No.: 38134

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material:	surPHace pHresh CONCENTRATE, Lot# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)
Appearance:	Slightly white colored, slightly cloudy (opaque) liquid
Dosage:	0.5 mL of undiluted test substance
Stability:	Room temperature, expiration date: expected to be stable for the duration of the study
Species:	Albino Rabbit, New Zealand White
Sex:	1 Male and 2 Females (nulliparous and non-pregnant)
Age:	Young adult at experimental start
Weight:	2.589-2.742 kg at experimental start
Source:	Robinson Services, Inc.
Acclimation:	13 or 19 days
Housing:	Singly housed in suspended stainless steel, perforated bottom cages. Litter paper was placed beneath the cage and was changed at least three times per week.
Food:	Harlan Teklad Global 16% High Fiber Rabbit Diet® 2031(approximately 150 grams/day) and a Premium Timothy Cube™ (Ontario Dehy, Inc.) were available to each rabbit.
Water:	Filtered tap water, available *ad libitum* except during exposure.
Experimental Conditions:	Temperature: 19-23^{0}C (normal range: 22^0 ± 3^{0}C) Humidity: 42-54% (normal range 30-70%) Light/Dark Cycle: 12 hours light/12 hours dark Air Exchanges: 12-13 per hour

Method: Primary Skin Irritation Study (single skin application, 0.5 ml of undiluted test substance)

Preparation and Selection of Animals: On the day prior to application of the test substance, the dorsal area of the trunk of a group of animals was clipped free of hair to expose test sites approximately 6 cm^2. On the day of dosing, prior to application, the animals were examined for health and the skin checked for any abnormalities. Three healthy naïve (not previously tested) animals without pre-existing skin irritation or defects were selected for testing.

Study Design: The primary skin irritation study was conducted in a step-wise fashion. Initially, only one rabbit was placed on test to determine the irritation/corrosion potential of the test substance. Three test sites, each at approximately 6 cm^2, were delineated on this rabbit. Based

upon the skin irritation results following application of test substance to each test site on this first animal (absence of corrosive effects up to 72 hours after patch removal), two additional animals were selected for further testing.

Application of Test Substance: Undiluted test substance (0.5 mL) was applied to each clipped skin site (three) on the first rabbit and covered with a 4-ply gauze pad, 1-inch x 1-inch. The pads and entire trunk of the animal were then wrapped with semi-occlusive, 3-inch Micropore™ tape to avoid dislocation of the three patches. An Elizabethan collar was placed on the rabbit after removal of the 3-minute patch and the animal was then returned to its designated cage. The pads were removed at the appropriate intervals (3 minutes, 1 hour and 4 hours) and the test sites cleansed with a 3% soap solution followed by tap water and a clean cloth paper towel to remove any residual test substance. All test sites were evaluated for corrosion (defined as full-thickness necrosis of the dose site) 30 to 60 minutes after each patch removal. Subsequent evaluations for corrosion were performed approximately 24, 48, and 72 hours after removal of the four-hour patch. Since there was no corrosion observed at any of the test sites for this animal, two additional rabbits (each with one patch only for a 4-hour exposure period), were selected for testing.

For the two additional rabbits, test substance was applied to the prepared test site (1) for a 4-hour exposure period. Following the 3-minute exposure period, the pad was removed and the site washed in the same manner as described above. For the 1-hour and 4-hour exposure periods, both the pads and collars were removed and the test sites also cleansed as described above.

Evaluation of Test Sites/Other Observations: Individual test sites were scored using the Draize scoring system (*J. Pharmacol. Exp. Ther. 1944; 82:377-390*) for erythema and edema formation and any other dermal effects at approximately 30-60 minutes, 24, 48 and 72 hours and at 7, 10 and 14 days after patch removal. The classification of irritancy was obtained by adding the average erythema and edema scores for the 30-60 minute, 24, 48 and 72-hour scoring intervals and dividing by the number of evaluation intervals (4). The resulting Primary Dermal Irritation Index (PDII) was classified according to the U.S. EPA Addendum 3 on data reporting to pesticide assessment guidelines; Dermal Irritation, January 1998. Statistical analysis was limited to the calculation of the mean irritation scores.

Body Weights and Cage-Side Observations: Individual weights of animals were recorded shortly before application of the test substance (initial) and at the completion of testing (terminal). The animals were also observed for signs of gross toxicity and behavioral changes at least once daily during the test period.

Study Termination: According to the study investigators, animals were humanely euthanized once testing was complete.

Results: All animals appeared active, healthy and gained weight during the study (Table 1). Apart from the dermal irritation noted below, there were no other signs of gross toxicity, adverse pharmacological effects, or abnormal behavior. No visible corrosion (necrosis) of the skin tissue was observed at the 3-minute, 1-hour and 4-hour test sites during the study.

Table 1. Individual Body Weights

Animal Number	Sex	Body Weight (kg)	
		Initial	Terminal
3501	M	2.589	2.641
3502	F	2.742	2.774
3503	F	2.652	2.811

Skin Irritation Incidence and Scores (Tables 2 and 3):

3-Minute Exposure Site (Animal Number 3501)

No dermal irritation was noted at the 3-minute exposure site for the first animal tested.

1-Hour Exposure Site (Animal Number 3501)

There was no edema observed at the 1-hour exposure site for the first animal tested. From 24 to 72 hours after patch removal, the treated site exhibited well-defined erythema. Blanching and desquamation were also observed at the treated site between 24 hours and Day 10. Very faint erythema was noted at the dose site from Day 7 to Day 14 (study termination).

4-Hour Exposure Site (Animal Numbers 3501, 3502 and 3503)

Over the first 72 hours after patch removal, three dose sites (one 4-hour exposure site per animal) exhibited very slight to moderate/severe erythema and very slight to slight edema. Blanching and/or areas of dark discoloration was also noted at all of the dose sites. The overall incidence and severity of irritation decreased gradually thereafter. Desquamation, small areas of superficial eschar, and/or hyperkeratosis were also noted at the dose sites between Days 7 and 14 with very slight erythema persisting at one dose site through Day 14 (study termination).

Table 2. Individual Skin Irritation Scores

Erythema/Edema								
Animal Number	Sex	30-60 min After Patch Removal	Hours After Patch Removal			Days After Patch Removal		
			24	48	72	7	10	14
3-Minute Skin Exposure								
3501	M	0/0	0/0	0/0	0/0	0/0	0/0	0/0
1-Hour Skin Exposure								
3501	M	0/0	2/0[1]	2/0[1]	2/0[1]	1/0[2]	1/0[2]	1/0
4-Hour Skin Exposure								
3501	M	1/1[1,3]	2/2[1,3]	3/1[1]	3/1[1]	2/0[2,4]	2/0[2,4]	1/0[2,4]
3502	F	2/1[1]	2/1[1]	2/1[1]	2/1[1]	1/0[5]	1/0[2]	0/0[2]
3503	F	1/1[1]	2/1[1,3]	2/1[1,3]	2/1[1,3]	1/0[5]	0/0[2]	0/0[2]
	Total	4/3	6/4	7/3	7/3	4/0	3/0	1/0
	Mean	1.3/1.0	2.0/1.3	2.3/1.0	2.3/1.0	1.3/0.0	1.0/0.0	0.3/0.0

[1]Blanching at dose site.
[2]Desquamation.
[3]Small areas of dark discoloration.
[4]Small areas of superficial eschar.
[5]Hyperkeratosis.

Table 3. Incidence and Severity of Skin Irritation*

Incidence of Irritation			
Time after Patch Removal	**Erythema**	**Edema**	**Severity Irritation (Mean Score)**
30-60 minutes	3/3	3/3	2.3
24 hours	3/3	3/3	3.3
48 hours	3/3	3/3	3.3
72 hours	3/3	3/3	3.3
Day 7	3/3	0/3	1.3
Day 10	2/3	0/3	1.0
Day 14	1/3	0/3	0.3

*Incidence and severity of skin irritation following a 4-hour skin exposure to the test substance.

The primary dermal irritation index (PDII) of 3.1 out of a possible 8.0 was obtained from observations at 1, 24, 48 and 72 hours after patch removal (Table 4). Based on a PDII of 3.1, surPHace pHresh CONCENTRATE is rated moderately irritating to the skin.

Table 4. Skin Irritation Scores for surPHace pHresh CONCENTRATE*

Irritation	**Time After Patch Removal**						
	30-60 min	**24 hours**	**48 hours**	**72 hours**	**Day 7**	**Day 10**	**Day 14**
Erythema**	1.3	2.0	2.3	2.3	1.3	1.0	0.3
Edema**	1.0	1.3	1.0	1.0	0.0	0.0	0.0
Total (PDI)[1]	**2.3**	**3.3**	**3.3**	**3.3**	**1.3**	**1.0**	**0.3**
[2]Primary Dermal Irritation Index (PDII) = 3.1							
Classification: Moderately Irritating							

*Skin irritation scores following a 4-hour skin exposure to the test substance.
**Average skin irritation scores for three rabbits.
[1]Total (PDI) = Total Primary Dermal Irritation = Average Erythema Scores + Average Edema Scores.
[2]Primary Dermal Irritation Index (PDII) = Sum of Total PDI for 30-60 minutes, 24, 48 and 72 hours divided by 4.

Under the conditions of the study, surPHace pHresh CONCENTRATE is assigned Toxicity Category III for primary dermal irritation based upon a PDII of 3.1.

Deviations from Guideline 870.2500: None

Summary:

1. **Toxicity Category**: III
2. **Classification**: Acceptable

DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)

Product Manager: 34
MRID No.: 49433207

Reviewer: Michelle Centra, Senior Pharmacologist
Study Completion Date: May 7, 2014
Report No.: 38135

Testing Laboratory: Product Safety Labs
Author: Daniel Merrill, B.S., MBA, Study Director

Quality Assurance (40 CFR §160): Included

Test Material: surPHace pHresh CONCENTRATE, Lot# 140121-4806 (19.33% Sulfuric Acid, as per the Certificate of Analysis dated February 13, 2014)
Appearance: Slightly white colored, slightly cloudy (opaque) liquid
Dosage: 0, 10, 25 and 50% undiluted test substance
Stability: Room temperature, expiration date: expected to be stable for the duration of the study

Species: Mouse, CBA/J
Sex: 33 Females (nulliparous and non-pregnant)
Animal Grouping: Total of 9 Groups
Number of Animals per Group:
- Preliminary Irritation (4 groups) - 2 per group
- Test (3 groups) - 5 per group
- Vehicle (Negative) Control - 5
- Positive Control - 5

Age: Preliminary Animals: Young adult, 9-10 weeks
Test and Control Animals: Young adult, 9-10 weeks at experimental start
Weight: Body Weight: 17.8-22.6 grams at experimental start (Day 1)
Source: The Jackson Laboratory.: Wilmington, MA
Acclimation: 14-15 days
Housing: Individually housed in plastic solid bottom cages during the dosing and resting phase of the study. After final weighing until sacrifice, animals were housed in their respective dose group in plastic cages with bedding which was changed at least once per week. Enrichment (e.g., nesting material) was placed in each cage.
Food: Harlan Teklad Global 16% Protein Rodent Diet® #2016, available *ad libitum*
Water: Filtered tap water, available *ad libitum*
Experimental Conditions:

Temperature	19-22^{0}C (normal range: 20^0 ± 3^{0}C)
Humidity	45-51% (normal range 30-70%)
Light/Dark Cycle	12 hours light/12 hours dark
Air Exchanges	13 per hour

Method: Local Lymph Node Assay in Mice for Skin Sensitization Potential

Positive

Control: Alpha-Hexylcinnamaldehyde (HCA); ≥ 97.9%; CAS #101-86-0; Lot# MKBL8623V; light yellow liquid; stored at room temperature; expiration date: January 13, 2015

Radioisotope: 3H-methyl Thymidine; Lot# 201403; Vial# 14-23; radiochemical purity ≥ 97%; specific activity: 20 Ci/mmol; radioactive concentration: 37 MBq/mL, 1.0 mCi/mL; stored refrigerated; expiration date: April 13, 2014

Selection of Animals/Clinical Observations/Body Weights: Animals were placed on study only if they showed no clinical signs of diseases or injury and only if their ears appeared normal (no abnormalities). Once selected for testing, all test, control and preliminary mice were observed daily for signs of mortality, gross toxicity, and/or behavioral changes. Individual body weights of test and control animals were recorded on study Day 1 (initial) shortly before test substance application and prior to IV injections on study Day 6. All test mice were euthanized via overdose of inhaled Isoflurane anesthetic on study Day 6.

Test Substance Preparation and Administration:

Preliminary LLNA Skin Irritation Test

For preliminary irritation testing of surPHace pHresh CONCENTRATE, three test substance concentrations and the vehicle were used in the mouse Local Lymph Node Assay (LLNA). Vehicle alone (N, N-dimethylformamide) or test substance at concentrations of 100% (undiluted; neat), 50% and 25% were applied to each ear to determine the highest achievable level that does not produce overt systemic toxicity or excessive local irritation. Dilutions (50% and 25%) were prepared as w/w mixtures in N, N-dimethylformamide on the day of application.

The three test substance concentrations and vehicle, each in a volume of 25 µL, were topically applied to the dorsal surface of each mouse ear on three consecutive days (study Days 1, 2 and 3). A micropipette was used to accurately deliver and evenly spread 25 µL applications over each dose site. No application was made on study Days 4 and 5. On study Day 6, the dose sites for each mouse were evaluated for local irritation (erythema and edema). The ears of each mouse in the four preliminary toxicity test groups (2 animals/group) were evaluated for erythema and edema at pre-dose on study Days 1, 2, 3 and 6 according to the Draize scoring system (*J. Pharmacol. Exp. Ther.; 82:377-390*). These preliminary irritation results in conjunction with daily observations for signs of toxicity and any pre-existing data (e.g., test substance toxicity, solubility, irritancy and viscosity) were used to select test substance concentrations to be used in the main study of the LLNA.

Main LLNA Skin Irritation Test

For testing in the main LLNA, 10%, 25% and 50% dilutions of surPHace pHresh CONCENTRATE were prepared as w/w mixtures in N, N-dimethylformamide. A single concentration of a 25% w/w mixture of Alpha-Hexylcinnamaldehyde (positive control) in N, N-dimethylformamide and a vehicle control containing only N, N-dimethylformamide were also prepared. Similar to the preliminary skin irritation test, all dosage preparations were prepared on the day of application.

Twenty-five healthy, naïve female animals (5 mice/group) were selected for LLNA testing and then distributed into test groups as presented in Table 1.

Table 1. Study Design

Group Number	Group Type	Dosage Preparations
11	Vehicle Control	N, N-Dimethylformamide
12	Positive Control	25% Alpha-Hexylcinnamaldehyde in N, N-Dimethylformamide
13	Test Substance	10% surPHace pHresh CONCENTRATE in N, N-Dimethylformamide
14	Test Substance	25% surPHace pHresh CONCENTRATE in N, N-Dimethylformamide
15	Test Substance	50% surPHace pHresh CONCENTRATE in N, N-Dimethylformamide

Prior to application of each dosage preparation, animal ears were evaluated for local irritation (erythema and edema) according to the Draize scoring system as cited above. Twenty-five µL of the appropriate test substance concentration, the positive control substance, or the vehicle alone was applied once per day to the dosrum of both ears of each mouse (50 µL of a specific dosage preparation/mouse) for three consecutive days (Days 1, 2 and 3). All ears were evaluated again for local irritation (erythema and edema) on study Day 6 (no dosage preparation application). Both application and ear evaluation protocols utilized in the main skin irritation test were performed in the same manner as described for the preliminary skin irritation test.

Injection of ^{3}H-methyl Thymidine: On Day 6 (three days after the final topical application), all Test Group and control animals were injected in the tail vein with 250 µL of phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-methyl Thymidine.

Lymph Node Assessment: Approximately five hours after the injection, animals were euthanized via overdose of inhaled Isoflurane and the draining auricular lymph nodes excised from all animals and pooled for each mouse. Lymph nodes were evaluated for each individual mouse. A single cell suspension of lymph node cells (LNC) was prepared in PBS by gently massaging the lymph nodes between frosted ends of two microscope slides over a collection vessel. The slides were then rinsed briefly with PBS into the vessel. The contents of the vessel were transferred to a centrifuge tube, washed with an excess of PBS and centrifuged for approximately 10 minutes, at 1800 rpm (relative centrifugal force of 489G). This wash and centrifugation was performed twice. The supernatant was decanted and discarded following each centrifugation. After the second wash, 5 mL of 5% trichloracetic acid (TCA) in distilled water was added to the precipitate and the tube briefly vortexed. The DNA was then precipitated in the TCA solution at 3.5-3.7^{0}C for approximately 18 hours. The tubes were centrifuged again and the supernatant discarded. The resulting precipitate was resuspended in 1 mL of the TCA solution and transferred to 10 mL of scintillation fluid. Incorporation of ^{3}H-methyl Thymidine was measured using B-scintillation counting and expressed as disintegrations per minute (DPM), minus background DPM.

Evaluation: The mean and standard deviation of the DPM values were calculated for each treatment group. A stimulation index (SI) was derived for each experimental group by dividing the mean DPM of each experimental group by the mean DPM of the vehicle control group, minus background DPM. Any test material that produces a SI $\geq$ 3 in the LLNA is positive for dermal sensitization potential (Kimber *et al.*, *Toxicology* 93, 13-31, 1994). The elicitation concentration value required to produce a three-fold increase in draining lymph-node cell proliferation activity

(EC3) was not calculated for the test substance since all dose levels tested in the LLNA induced a stimulation index (SI) less than 3.0.

Statistical Analysis: Statistical analysis was performed and significance was judged at $p<0.05$. The treated groups and negative vehicle control group were compared using a One-Way Analysis of Variance (ANOVA), followed by comparison of the treated groups to control by Dunnett's t-test for multiple comparisons. Where variances are considered significantly different by Bartlett's test, groups were compared using a non-parametric method (Kruskal-Wallis non-parametric analysis of variance followed by Dunn's test) (INSTAT Biostatistics, Graph Pad Software, San Diego, CA). Outlier analysis was conducted (Grubbs, *Technometrics*, Vol. 11, No. 1, 1969, pp. 1-21).

Results:

Individual Body Weights (Table 2) - Most animals either maintained their initial body weight or gained body weight between study Day 1 and 6. Although four animals lost weight during the study, the observed body weight loses were slight and not considered toxicologically significant. All animals appeared healthy and active during the study.

Table 2. Individual Body Weights

Animal Number	Group	Sex	Body Weight (g)	
			Day 1	Day 6
3651	11 Vehicle Control (N, N-Dimethylformamide)	F	18.7	18.4
3652		F	19.8	20.4
3653		F	19.7	20.8
3654		F	20.4	21.4
3655		F	18.1	17.6
3656	12 Positive Control (25% HCA in N, N-Dimethylformamide)	F	18.3	19.5
3657		F	17.8	18.4
3658		F	19.6	20.2
3659		F	19.6	19.6
3660		F	18.0	19.3
3661	13 10% surPHace pHresh CONCENTRATE (in N, N-Dimethylformamide)	F	18.8	19.6
3662		F	18.6	19.9
3663		F	18.4	19.9
3664		F	20.9	20.1
3665		F	20.7	21.0
3666	14 25% surPHace pHresh CONCENTRATE (in N, N-Dimethylformamide)	F	19.2	19.3
3667		F	18.6	19.1
3668		F	19.6	19.8
3669		F	18.2	18.8
3670		F	19.2	19.6
3671	15 50% surPHace pHresh CONCENTRATE (in N, N-Dimethylformamide)	F	19.0	19.1
3672		F	19.3	20.1
3673		F	19.9	21.2
3674		F	22.6	22.1
3675		F	20.0	20.7

Main Skin Irritation Test - Results are presented in Table 3 and are summarized below.

Table 3. Individual Dermal Irritation Scores

Animal Number	Sex	Erythema/Edema							
		Days							
		1		2		3		6	
		Left	Right	Left	Right	Left	Right	Left	Right
Group 11 – Vehicle Control, N, N-Dimethylformamide[1]									
3651	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3652	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3653	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3654	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3655	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Group 12 – Positive Control, 25% Alpha-Hexylcinnamaldehyde in N, N-Dimethylformamide[2]									
3656	F	0/0	0/0	1/0	0/0	1/1	1/0	1/1[4]	1/0[4]
3657	F	0/0	0/0	1/0	1/0	1/0	1/0	1/1[4]	1/1[4]
3658	F	0/0	0/0	0/0	1/0	1/0	1/0	1/1[4]	1/0[4]
3659	F	0/0	0/0	1/0	1/0	1/0	1/1	1/1[4]	1/1[4]
3660	F	0/0	0/0	1/0	0/0	1/0	1/0	1/1[4]	1/0[4]
Group 13 – 10% SurpHace pHresh CONCENTRATE in N, N-Dimethylformamide[3]									
3661	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3662	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3663	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3664	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]
3665	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]
Group 14 – 25% SurpHace pHresh CONCENTRATE in N, N-Dimethylformamide[3]									
3666	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]
3667	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3668	F	0/0	0/0	0/0	0/0	0/0	0/0	1/0[4]	0/0[4]
3669	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3670	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
Group 15 – 50% SurpHace pHresh CONCENTRATE in N, N-Dimethylformamide[3]									
3671	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]
3672	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3673	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
3674	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]
3675	F	0/0	0/0	0/0	0/0	0/0	0/0	0/0[4]	0/0[4]

[1]25 µL of N, N-Dimethylformamide applied to each ear (50 µL total/rabbit).
[2]25 µL of a 25% w/w mixture of alpha-hexylcinnamaldehyde in N, N-Dimethylformamide applied to each ear (50 µL total/rabbit).
[3]25 µL of the test substance applied as a w/w mixture in N, N-Dimethylformamide applied to each ear (50 µL total/rabbit).
[4]Desquamation at dose site.

Group 11 - No dermal irritation was observed for any vehicle control sites.

Group 12 - Very slight erythema (score of 1) was evident at seven positive control sites on Day 2, and at all positive control sites on Days 3 and 6. Very slight edema (score of 1) was present at two positive control sites on Day 3 and at seven positive control sites on Day 6. Desquamation was present at all positive control sites on Day 6.

Group 13 - No dermal irritation was observed for any of the test sites. Desquamation was present at eight test sites on Day 6.

Group 14 - Very slight erythema (score of 1) was evident at one test site on Day 6. Desquamation was present at nine test sites on Day 6.

Group 15 - No dermal irritation was observed for any of the test sites. Desquamation was present at five test sites on Day 6.

Stimulation Index - Results are presented in Table 4 and are summarized below.

Table 4. Stimulation Index (SI)

Animal Group	Test Substance Concentration	Number of Animals	Average Net DPM (minus background DPM[1,2])	Standard Deviation	SI[3]	Sensitization Response (SI > 3)
Vehicle Control (Group 11)	N, N-DMF	5	640.92	203.67	-	N/A
Positive Control (Group 12)	25% HCA in N,N DMF	5	2104.73**	339.36	3.28	Positive (validation test)
Test Substance (Group 13)	10% surPHace pHresh CONCENTRATE in N, N-DMF	5	695.97	103.02	1.09	Not a sensitizer
Test Substance (Group 14)	25% surPHace pHresh CONCENTRATE in N, N-DMF	5	858.25	267.45	1.34	Not a sensitizer
Test Substance (Group 15)	50% surPHace pHresh CONCENTRATE in N, N-DMF	5	619.28	153.50	0.97	Not a sensitizer

N/A = Not applicable; DPM = Disintegrations per minute; N, N-DMF = N, N-Dimethylformamide; HCA = Alpha-Hexylcinnamaldehyde; SI = Stimulation Index.
[1]Background DPM = 69.44.
[2]Values analyzed for outliers.
[3]Stimulation Index (SI) for each experimental group was derived by dividing the average DPM of an experimental group by the average DPM of the vehicle control group, minus background DPM.
**Statistically significant difference from vehicle control at $p < 0.01$ by Dunnet Multiple Comparisons Test.

Treatment of mice with 10%, 25% and 50% of surpHace pHresh CONCENTRATE resulted in stimulation index values of 1.09, 1.34 and 0.97, respectively. As a stimulation index (SI) of less than 3.0 was observed in all treatment groups, the test substance was not considered positive for a dermal sensitization potential. The positive control (HCA) at 25% produced a moderate dermal sensitization response in mice (SI = 3.28) and therefore, validated conduct of the LLNA test system with surpHace pHresh CONCENTRATE.

Conclusion: Based on the results of this study, surpHace pHresh CONCENTRATE is not considered to be a contact dermal sensitizer at concentrations of less than or equal to 50% in the LLNA.

Deviations from Guideline 870.2600: An initial test and a repeat test of the positive control substance, 25% Aplha-hexylcinnamaldehyde in N, N-dimethylformamide, failed to validate the method used by Product Safety Labs to assess the dermal sensitization potential of the test substance using the LLNA; the positive control substance did not elicit a positive response. study was repeated third time. Since the third test showed the positive control substance to be a moderate skin sensitizer, LLNA testing to assess potential skin sensitization of surpHace pHresh

CONCENTRATE was considered valid. Therefore, this deviation in protocol did not affect interpretation of the LLNA study results.

Summary:

1. **Under the conditions of the LLNA, surpHace pHresh CONCENTRATE is not a skin sensitizer.**
2. **Classification:** Acceptable